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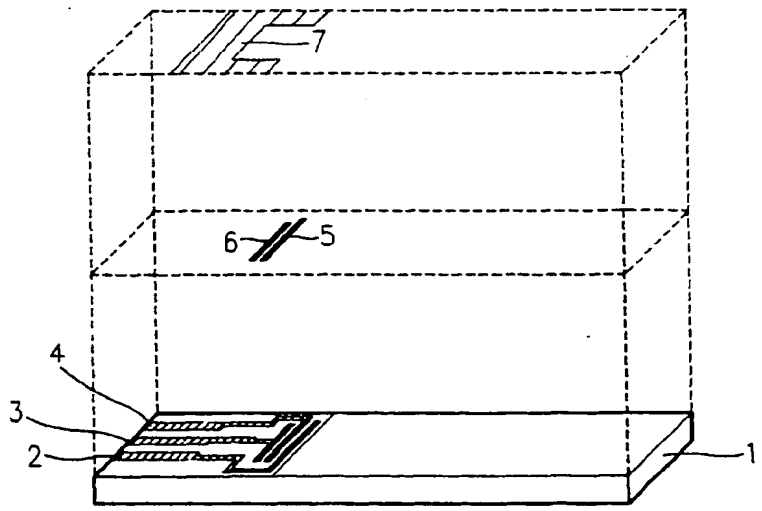
(54) Electrochemical immunobiosensor

(57) The present invention relates to an electrochemical immunobiosensor which comprises an electrode part where a plurality of electrodes are formed on an insulating base plate and a part of the electrode is exposed by selectively forming an insulating layer on the electrode; and a matrix part where a portion absorbed with analyte-cytolytic agent conjugates, a portion with an immobilized recepter which binds with the analyte, and a portion fixed with electroactive species-containing liposomes, to perform the functions of liposome-based signal amplification and immunochromatography; and said electrode portion exposed in the electrode part and the liposome portion in the matrix are integrally combined.

FIG.1



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Description

FIELD OF THE INVENTION

The present invention relates to an electrochemical immunobiosensor. More specifically, the present invention relates to an immunobiosensor which can quantitatively analyse the concentration of physiologically important materials present in a sample by measuring the result of immunochemical reaction between antigen and antibody electrochemically.

BACKGROUND OF THE INVENTION

As the reaction occurs with high specificity and strong binding force in an antigen-antibody reaction, the immunobiosensor using the reaction has a lot more excellent characteristics than other sensors in assaying the physiologically important materials. As conventional immunobiosensors using optical phenomenon, a method measuring the light intensity altering with the result of interaction between an immobilized antibody at the end of optical fiber and an analyte (antigen) [Anderson G.P. et al., IEEE Trans. Biomed. Eng. 1994, 41, 578-584]; a method which comprises fixing of an antibody on a piezoelectric element and altering the change of mass, induced by binding thereof with an analyte(antigen), into an electric signal [König. B. et al., Anal. Chem. 1994, 66, 341-344]; a method detecting the change of surface plasom resonance phenomenon occuring at the liquid interface with a metal by binding of an immobilized antibody on the metal with an analyte (antigen) [Severs, A.M. et al., Biosensors & Bioelectronics, 1993, 8, 365-370], or the like have been reported.

As an immunobiosensor using an electrochemical method, a method which comprises generating electroactive species by using conventional immunoassay such as enzyme-linked immunoSorbent assay(ELISA) or metal ion labelling, separating thereof to measure electrochemically [Hayes, F.J. et al., Anal. Chem. 1994, 66, 1860-1865].

Liposome immunoassay methods in which a labelled material is encapsulated by the liposome and the liposome is ruptured after the completion of the immunochemical reaction to release the labelled material so that a signal related to the concentration of the analyte are recently in the spotlight. Liposomes have been investigated as a drug delivery system as their main component is phospholipid and have a property of capturing a hydrophilic or hydrophobic material or adjusting the passage of the entrapped material dependent upon the characteristics of the bilayer, as the properties of the cell membranes. A variety of researches for applying these liposomes in immunoassays have been performed recently, however, few examples where liposomes applied in a sensor have been reported up to the present [Richard, A. et al., Biosensors & Bioelectronics, 1993, 8, xiii-xv].

Conventional immunobiosensors using optical phenomenon have disadvantages in that the measuring system is complicated and high cost is required therefor. In contrast, the immunobiosensors to which the principles of electrochemistry have been applied have very simple measuring system, and the process for production of electrode is also simple.

Though a conventional immunoassay using electrochemical measuring system has been reported [Athey, D. et al., Biosensors & Bioelectronics, 1993, 8, 415-419], it requires long time for the assay because the procedure is using the conventional immunoassay system as it is and electrochemical measurement is used in only the final assay stage, and the assay is not practically simple because a variety of complicated stage during the assay. Generally, an immunochemical reaction such as antigen-antibody reaction requires much time because of the characteristics of the reaction per se.

The reason why development of such electrochemical immunobiosensor in its true meaning is inactive comes from a technical difficulties in inducing a electroactive species from antigen-antibody binding itself. In case of an electrochemical sensor using an enzyme, electroactive species are readily formed on an electrode as a result of the enzyme reaction on the electrode and the material is oxidized or reduced to produce electrical current, while in case that an antibody on an electrode is reacted with an analyte (antigen), no electroactive species is produced. In order to overcome such problem, attemps for preparation of an antibody having enzyme activity have been reported.

An immunoassay using liposome and application thereof as a sensor are also complicated, because in case of immunoassay, required material for each step should be sequentially added, while in case of recently introduced immunobiosensor using liposomes, liposomes as well as a sample should be added so that the user must treat additional material containing liposomes as well as the sensor itself at the time of using thereof. Besides, liposomes must be ruptured by adding additional material in order to perform an electrochemical measurement, whereby the method is not simple.

An immunobiosensor using immunochemical reaction such as antigen-antibody reaction can not be produced in a strip type as that of conventional thick-film enzyme sensor. This is because no electroactive species occur as a result of immunochemical reaction itself; and in case of so called "sandwich assay" [where the antibody is fixed on the upper part of the electrode and then the analyte (antigen) in the sample is bound to the antibody, and it is reacted with the second antibody combined to an enzyme or electroactive species], the sensor must be washed during the assay and auxiliary reagents are needed.

SUMMARY OF THE INVENTION

It would therefore be desirable to overcome the above mentioned problems and to provide an electrochemical immunobiosensor which can directly measure electrochemically the result of antigen-antibody reaction, by taking advantage of liposomes which can comprise excess amount of electroactive species as signal-generating source, and principles of electrochemistry having a simple measuring system.

It would also be desirable to provide a disposable immunobiosensor of strip type which can simply assay in a short time by just dropping a sample onto the sensor, without complicated multi-step operation during the assay.

It would also be desirable to provide a disposable immunobiosensor having a structure wherein a chromatographic matrix (which causes immunochemical reaction by developing the sample) is combined with an electrode of strip type, in order to quantify the concentration of the material to be assayed in the sample by contacting and reacting the sensor with the sample once without any additional auxiliary reagent.

It would also be desirable to provide an electrochemical immunobiosensor in which an immunochemical reaction occurs while the sample is developed along the chromatographic matrix, having a structure wherein an analyte-cytolytic agent conjugates, an antibody(or antigen) which selectively combines with the analyte, and liposomes containing electroactive species are adsorbed or immobilized in a specific site respectively.

It would also be desirable to provide an electrochemical immunobiosensor which can accurately measure the physiologically important analytes at a high sensitivity by integrating the matrix and the electrode prepared on the insulating base plate so that the electroactive species released by the liposomelysis can be effectively oxidized or reduced by using the principles of generation of signal from electroactive species-containing liposomes.

It would also be desirable to provide a multi-immunobiosensor for measuring multicomponents.

An electrochemical immunobiosensor satisfying the desires described above may comprise an electrode part where a plurality of electrodes are formed on an insulating base plate and a part of the electrode is exposed by selectively forming an insulating layer on the electrode; and a matrix part where a portion absorbed with analyte-cytolytic agent conjugates, a portion immobilized with a recepter which binds with the analyte, and a portion immobilized with electroactive species-containing liposomes, to perform the functions of liposome-based amplification signal and immunochromatography; and said electrode portion exposed in the electrode part and the liposome portion in the matrix are integrated.

In a currently preferred embodiment the multi-immunobiosensor comprises overlapping a plurality of the electochemical immunobiosensors which comprises an electrode part where a plurality of electrodes are formed on an insulating base plate and a part of the electrode is exposed by selectively forming an insulating layer on the electrode; and a matrix part where a portion absorbed with analyte-cytolytic agent conjugates, a portion immobilized with a recepter which binds with the analyte, and a portion immobilized with electroactive species-containing liposomes; and said electrode portion exposed in the electrode part and the liposome portion in the matrix are integrated, with comparative sensor which generates reference signal to enable simultaneous measurement of physiologically important multi-component species.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a perspective view of a electrode system which is composed of 3-electrode system of immunobiosensor embodying the present invention.

FIG. 2 is a plane view of a electrode system which is composed of 3-electrode system of immunobiosensor embodying the present invention.

FIG. 3 is a perspective view of a electrode system which is composed of 2-electrode system of immunobiosensor embodying the present invention.

FIG. 4 is a plane view of a electrode system which is composed of 2-electrode system of immunobiosensor embodying the present invention.

FiG. 5 is a structural view of matrix part of immunobiosensor embodying the present invention.

FIG. 6 is s perspective view of immunobiosensor embodying the present invention.

FIG. 7 (A) - (F) illustrates the principle of generating signal of the immunobiosensor embodying the present invention.

FIG. 8 is a perspective view of the immunobiosensor for measuring surface antibody and surface antigen of Hepatitis B virus concurrently according to one embodiment of the present invention.

FIG. 9 is a perspective view of multi-immunobiosensor for measuring multi-component according to another embodiment of the present invention.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENT

An embodiment of the present invention is described by referring to the figures attached.

In a currently preferred embodiment the electrochemical immunobiosensor is composed of electrode part and chromatography(or capillary) matrix, whrein electrode part is formed on an insulating base plate using thick-film technology, and immunochemical reaction occurs by developing the sample by a chromatographic principle or capillary action when sample is applied.

The structure of electrochemical immunobiosensor is explained with reference to FIGs. 1 to 6.

Electrodes, same as in a known sensor using electrochemical principle, have a 2-electrode system copmosed of a reference electrode and a working electrode, or a 3-electrode system wherein a counter electrode is introduced thereto. Oxidation or reduction of electroactive species occurs on the working electrode under a certain applied potential with respect to the reference electrode.

As shown in FIG.1 and 2, electrode system part(9) consisting of a 3-electrode system is constructed of a conduction path(2,3,4) printed with silver(Ag) paste, a working electrode(5) printed with carbon paste, a counter electrode(6), an Ag/AgCl reference electrode(8) and an insulating layer(7) formed on an insulating base plate (1) made of a polymer such as polyester, polyvinyl chloride or polycarbonate.

FIG. 2 is a plane view of a electrode system part(9) which is constructed on an insulating base plate.

On the other hand, the electrode system consisting of 2-electrode system comprises only working electrode (5') and reference electrode(8') as shown in FIGs. 3 and 4.

In general, the material for preparing the working electrode includes carbon, platinum, gold, ruthenium dioxide, or the like.

The materials for constructing the matrix part(15) which generates an immunochemical reaction include filter paper, nitrocellulose, polystyrene, polyvinyl, activated nylon film, or the like, as illustrated in FIG. 5. On the right upper side of the matrix part(15), i.e. assay starting point, an analyte-cytolytic agent conjugate is absorbed, while on the central upper side. i.e. competition point(13), an antibody(or antigen) which can binds to the analyte is strongly bound so that it cannot move, and on the left upper side, i.e. signal-generating point, are immobilized liposomes containing electroactive species such as ferrocene derivatives(e.g. ferrocene, dimethylferrocene), potassium ferrocyanide, viologen derivatives, 2,6-dichlorophenolindophenol (DCPIP), p-aminophenol, tetrathiafulvalene (TTF), tetracyanoquinodimethane (TCNQ), phenazine methosulphate (PMS), Meldola's blue (7-dimethylamino-1,2-benzophenoxazine), 1,2-benzophenoxazine-7-one (BPO), thionin, or the like.

The matrix part(15) has the function of generating immunochemical reaction as well as providing electrolytes in the sample to the electrode part(10) by mobilizing the analyte-cytolytic agent conjugates absorbed on the matrix.

As shown in FIG. 5, the matrix part(15) and electrode system(9) having characteristics mentioned above are integrally combined to the electrode portion (10) exposed by an insulating layer on the base electrode, and the portion(14) to which liposomes containing electroactive species are immobilized on the matrix part (15), to complete the immunobiosensor (19).

FIG. 6 is a perspective view of the immunobiosensor completed by the processes mentioned above, and symbol 18 shows sample application part.

The principles of generating signals by the immunobiosensor are explained with reference to FIG. 7.

In case that the analyte is an antigen, a predetermined amount of a liquid sample containing the analyte is dropped on the sample application part(18) on the sensor [FIG. 7]. Then, the sample is mixed with the analyte-cytolytic agent conjugates absorbed on the assay starting point(12) of the matrix part(15) as shown in FIG. 7, and starts to move with the analyte in the sample by using the liquid in the sample as a carrier, toward the electrode by chromatographic principle [FIG. 7(C)]. In the competition point (13) to be reached at this time, an antibody which specifically binds to the analyte is immobilized on the matrix. To occupy the binding site for antigen-antibody, the analyte and analyte-cytolytic agent conjugates in the sample compete each other [FIG.7 (D)].

Thus, if a large amount of the analyte is present in the sample, much analyte-cytolytic agent pass through the competition point(13) to reach the liposomes on the signal-generating point(14) just over the electrode, to cause the liposome lysis. [Fig.7(E)]. As a result of the immunochemical reaction, electroactive species in the liposomes are released proportional to the concentration of the analyte present in the sample solution, and the released electroactive species diffuse and reach to the electrode part(10) [FIG.7(F)]. As the current is generated between the working electrode(5) and the counter electrode(6) upon oxidizing or reducing the electroactive species on the working electrode applied with a certain potential compared to the reference electrode, a current value proportional to the concentration of the analyte present in the sample solution can be obtained.

On the contrary, if the analyte is an antibody, an antigen which specifically binds with the analyte is immobilized on the competition point(13) on the matrix in FIG. 7, so that an assay of the analyte (antibody) can be performed by the same principle.

As described above, the embodiments of the invention may be applied in all immunoassay using the affinity between ligand and receptor such as conventional antigen-antibody reaction [e.g. are biotine-avidin, immunoglobulin G-protein A, hormone-hormone receptor, DNA-DNA, RNA-RNA and drug-drug receptor].

Here-in-after, an immunobiosensor which enables to measure viruses such as Hepatitis virus and AIDS virus, pathogenic microbes such as *Salmonella* and *Legionella,* proteins such as immunoglobulin, lipoprotein such as HDL and LDL, hormones such as human growth hormone and TSH(Thyroid Stimulating Hormone), antibiotics such as gentamicine and tobramicine, and drugs such as digoxin and theophylline and a multi-immunobiosensor which enables to measure multicomponents concurrently will be described in more detail by reference to the following examples.

Example 1 : Immunobiosensor for measuring surface antigen of Hepatitis B virus(HBsAg)

Surface antigen of Hepatitis B virus(HBsAg) is a marker for determination whether A person has been infected by Hepatitis B virus or not.

At first, the fabrication for base electrode is carried out in the following order:

As in FIG. 1 and 2, a polyester insulating base plate (1) with 0.3 mm thickness is washed with aceton and silver(Ag) paste is printed thereon and conduction path (2,3,4) and connection paths are formed by heating at 110 °C for 10 minutes. Carbon paste is printed on a working electrode(5) and counter electrode(6) portions and heat-treated as above. And after insulating layer(7) is formed by printing insulating paste thereon, electrode portion(10) exposed by insulating layer is treated with 100 mM $FeCl_3$ solution for 1 minutes and remaining $FeCl_3$ solution is washed off by distilled water to form a Ag/AgCl reference electrode(8). Thus, electrode system composed of a working electrode(5), a counter electrode(6) and a reference electrode(8) has been completed.

The fabrication of electroactive species-containing liposome is carried out in the following order:

The electroactive species-containing liposome is prepared in the form of LUV(Large Unilamella Vesicle) or MLV(Multilamella Lipid Vesicle) by general methods. Namely, phospholipid such as DPPC(dipalmitoylphosphatidylcholine), DPPG(dipalmitoylphosphatidyl glycerol) and cholesterol is dissolved in proper ratio in organic solvent such as chloroform and the solution is placed in a glass vessel for evaporation under reduced pressure to form a lipid film. A solution of 100 mM potassium ferrocyanide dissolved in phosphate buffer (pH 7.4) is added thereto and the mixed solution is stirred vigorously to form a opaque liposome phase with particle sizes of 100 -1000 nm.

An chromatographic matrix is fabricated as in follows:

A paper for chromatography such as cellulose paper is cut into 6 mm X 50 mm size and matrices are pretreated by dipping into 0.1 M sodium carbonate buffer wherein 2.5 g of casein is dissolved, and washed with distilled water repeatedly and then dried. As in FIG. 5, potassium ferrocyanide-containing liposome is immobilized on a signal generating point(14) in pretreated matrix part(15) and an antibody binding with target antigen (HBsAg) is immobilized on a competition point(13). And a conjugate wherein a cytolytic agent, melittin, is bound to the antigen(HBsAg) is absorbed to the assay starting point(12).

As shown in FIG. 5, matrix part(15) and the electrode system(9) fabricated as above are combined together so as to electrode portion(10) exposed by the insulating layer on base electrode being accord with the portion(14) wherein liposome of matrix part(15) is immobilized, using doubled-sided adhesive tape(16) and a polyester upper supporting layer(17) to complete immunobiosensor(19) for measuring surface antigen of Hepatitis B virus.

And at measurement, sample blood is dropped on sample application part(18) and melittin-HBsAg conjugate absorbed at the assay starting point(12) of the matrix part(15) begins to move with sample by develpoing through the matrix. As approaching to the competition point(13), a surface antigen(HBsAg), measured species in sample, competes with a melittin-HBsAg conjugate for binding and as a result, melittin, cytolytic moiety of melittin-HBsAg conjugate reaching at signal generating point(14) through the competition point(13), rupturs liposome and potassium ferrocyanide contained in liposome will be released. At this time, on the 200 mV applied working electrode(5) compared to the reference electrode(8), potassium ferrocyanide is oxidized($Fe(CN)_6^{-4}$ ---> $Fe(CN)_6^{-3} + e^-$) and the current is resulted in proportion to HBsAg concentration in sample.

Example 2 : Immunobiosensor for measuring surface antibody of Hepatitis B virus(anti-HBsAg)

Surface antibody of Hepatitis B virus is a marker for determination whether antibody for Hepatitis B virus, in other words, immunity has been formed or not.

The fabrications of base electrode and the preparation of electroactive species-containing liposome are carried out in accordance with the same procedure as in Example 1.

The fabrication of matrix for immunochemical reaction is carried out in the following order:

A paper for chromatography such as cellulose paper is cut into 6 mm X 50 mm size and matrices are pretreated by dipping into 0.1 M sodium carbonate buffer wherein 2.5 g of casein is dissolved, and washed with distilled water repeatedly and then dried. The potassium ferrocyanide-containing liposome is immobilized on a signal generating point(14) in pretreated matrix part(15) and an antigen binding with target antibody is immobilized on a competition point(13). And a conjugate wherein a cytolytic agent, melittin, is bound to the antibody is absorbed to the assay starting point(12).

As in FIG. 5, chromatographic matrix part(15) and the electrode system(9) fabricated as above are combined together so as to electrode portion(10) exposed by the insulating layer on base electrode being accord with the portion(14) wherein liposome of matrix part(15) is immobilized, using doubled-sided adhesive tape(16) and a polyester upper supporting layer(17) to complete the immunobiosensor(19) for measuring surface antibody of Hepatitis B virus.

And at measurement, sample blood is dropped on sample application part(18) and melittin-surface antibody conjugate absorbed at the assay starting point(12) of the matrix part(15) begins to move with sample by developing through the matrix. As approaching to the competition point(13), surface antibody(anti-HBsAg), the meas-

ured species in sample, competes with melittin-surface antibody conjugate for binding and as a result, melittin, cytolytic moiety of melittin-surface antibody conjugate reaching at signal generating point(14) through the competition point(13), rupturs liposome and potassium ferrocyanide contained in liposome will be released. At this time, on the 200 mV applied working electrode(5) compared to the reference electrode(8), potassium ferrocyanide is oxidized($Fe(CN)_6^{-4}$ ---> $Fe(CN)_6^{-3} + e^-$)and the current is resulted in proportion to surface antibody(anti-HBsAg) concentration in sample.

Example 3 : Immunobiosensor for measuring theophylline

The fabrication of base electrode is carried out in accordance with the same procedure as in Example 1.

The fabrication of electroactive species-containing liposome is carried out in the following order:

The electroactive species-containing liposome is prepared in forms of LUV(Large Unilamella Vesicle) or MLV(Multilamella Lipid Vesicle) by general methods. Namely, phospholipid such as DMPC(dimyristoylphosphatidylcholine) and cholesterol is dissolved in proper ratio in organic solvent such as chloroform and the solution is placed in a glass vessel for evaporation under reduced pressure to form a lipid film. A solution of 100 mM ferrocene dissolved in phosphate buffer (pH 7.4) is added thereto and the mixed solution is stirred vigorously to form a opaque liposome phase with particle sizes of 100 - 1000 nm.

The fabrication of matrix for immunochemical reaction is carried out in the following order:

The ferrocene-containing liposome is immobilized on a signal generating point(14) in pretreated nitrocellulose matrix part(15) and an antibody binding with target theophylline(anti-theophylline) is immobilized on the competition point(13). And a conjugate wherein a cytolytic agent, phospholipase C, is bound to theophylline is absorbed to the assay starting point(12).

As in FIG. 5, the matrix part(15) and the electrode system(9) fabricated as above are combined together so as to electrode portion(10) exposed by the insulating layer on base electrode being accord with the portion (14) wherein liposome of matrix part(15) is immobilized, using doubled-sided adhesive tape(16) and a polyester upper supporting layer(17) to complete the immunobiosensor(19) for measuring theophylline.

At measurement, sample blood is dropped on sample application part(8) and then, sample and phospholipase C-theophylline conjugate begin to move by developing through the matrix. As approaching to the competition point(13), theophylline, the analyte, competes with phospholipase C-theophylline conjugate for binding and as a result, phospholipase C, cytolytic moiety of phospholipase C-theophilline conjugate reaching at the signal generating point(14) through the competition point (13), rupturs liposome and ferrocene contained in liposome will be released. At this time, on the 150 mV applied working electrode(5) compared to reference electrode(8), ferrocene is oxidized(ferrocene --> ferricinium + e^-) and the current is resulted in proportion to theophilline concentration in sample.

Example 4 : Immunobiosensor for measuring human growth hormone (HGH)

The fabrication of base electrode is carried out in accordance with the same procedure as in Example 1.

The fabrication of electroactive species-containing liposome is carried out in the following order:

The electroactive species-containing liposome is prepared in the form of LUV(Large Unilamella Vesicle) or MLV(Multilamella Lipid Vesicle) by general methods.

Namely, phospholipid such as DMPC(dimyristoylphosphatidylcholine) and cholesterol is dissolved in proper ratio in organic solvent such as chloroform and the solution is placed in a glass vessel for evaporation under reduced pressure to form a lipid film. A solution of 100 mM DCPIP(2,6-dichlorophenolindophenol) dissolved in phosphate buffer (pH 7.4) is added thereto and the mixed solution is stirred vigorously to form a opaque liposome phase with particle sizes of 100 -1000 nm.

The fabrication of matrix for immunochemical reaction is carried out in the following order:

The DCPIP-containing liposome is immobilized on the signal generating point(14) in pretreated nitrocellulose matrix part(15) and an antibody(anti-HGH) binding with target human growth hormone(HGH) is immobilized on the competition point(13). And a conjugate wherein a cytolytic agent, phospholipase C, is bound to human growth hormone is absorbed to analysis beginning point(12).

As in FIG. 5, the matrix part(15) and the electrode system(9) fabricated as above are combined together so as to electrode portion(10) exposed by the insulating layer on base electrode being accord with the portion (14) wherein liposome of matrix part(15) is immobilized, using doubled-sided adhesive tape(16) and a polyester upper supporting layer(17) to complete the immunobiosensor(19) for measuring HGH.

At measurement, sample blood is dropped on sample application part(18) and then, sample and phospholipase C-HGH conjugate begin to move by developing through the matrix. As approaching to the competition point(13), HGH; competes with phospholipase C- HGH conjugate for binding and as a result, phospholipase C, cytolytic moiety of phospholipase C-theophilline conjugate reaching at the signal generating point(14) through the competition point(13), rupturs liposome and DCPIP contained in liposome will be released. At this time, on the 150 mV applied working electrode(5) compared to the reference electrode(8), DCPIP is reduced and the current is resulted in proportion to HGH concentration in sample.

Example 5. Immunobiosensor for concurrent measuring surface antibody and surface antigen of Hepatitis B virus

The immunobiosensor for measuring surface antigen of Hepatitis B virus(HBsAg) and the immunobiosensor for measuring surface antibody of Hepatitis B virus (anti-HBsAg) fabricated respectively as in above Example 1 and 2, are bound by overlapping as in FIG. 8 to complete a immunobiosensor for measuring surface antigen and surface antibody of Hepatitis B virus concurrently.

Example 6. Immunobiosensor for measuring multicomponents

Similar to above Example 5, the individual immunobiosensors(22-25) fabricated respectively as in above Example 1 to 4, are bound by overlapping as in FIG. 9 to complete a multi-immunobiosensor for measuring multicomponents. At this time, identical electroactive species-containing liposome is used for individual immunobiosensors(22-25), and control sensor(26) generating a kind of reference signal is overlapped thereon to fabricate the multi-immunobiosensor for measuring multicomponents. Hence, electrode signal output from individual immunobiosensors(22-25) will be amplified differentially from background signal due to no relevance to immunochemical reaction. And detection of signal without interference effect can be obtained. In control sensor (26) generating reference signal, electroactive species-containing liposome is immobilized only at the signal generating point(14) on nitrocellulose matrix part(15). Therefore, in case that a cytolytic agent is present in sample or in case that liposome is ruptured without immunochemical reaction, accurate measurement will be possible. In sample measurement, measurement circuit connection part(27) is connected to a measurement apparatus and sample is applied to react the sample contact portion(28) of the multi-immunobiosensor for the measuring multicomponents.

As described above, because electrochemical immunobiosensor embodying the present invention takes advantages of liposome wherein electroactive species as a signal-generating material are contained in excess amount and together with the measurement system due to the simple electrochemical principle than any other methods, the described immunobiosensor measures the result of antigen-antibody reaction directly.

According to the immunobiosensor described, all analyses are performed in a short time by simply placing the sample on the specific portion of the sensor and thus, it will be an excellent immunoassay means that can be applied in quick and easy way in various clinic field.

Also because a disposable strip-type immunobiosensor embodying the present invention is packaged and supplied in a dry form, it has an advantage of enhancing the user's convenience.

The disposable strip-type immunobiosensor embodying the present invention provides an methods that patient's health condition is diagnosed directly on the spot in clinic of a private hospital as well as a general hospital and also greatly serves for national health and welfare due to a rapid prescription. Therefore, the immunobiosensor embodying the present invention has an advantage that the present product differs from typical analysis system taking several hours.

The matrix embodying the invention has a structure that can be characterized in that a measuring analyte analyte-cytolytic agent conjugate, an antigen(or an antibody) selectively binding with the analyte and an electroactive species-containing liposome are respectively absorbed or fixed onto specific portion of chromatographic matrix phase wherein immunochemical reaction used in the present invention is carried out. Hence, the described embodiment has an advantage applicable to general ligand-receptor pair, such as immunoglobulin G-protein A, hormone-hormone receptor, DNA-DNA, RNA-RNA and drug-drug receptor as well as typical antigen-antibody reaction.

Therefore the described embodiment has an advantage to fabricate a immunobiosensor which enables to measure viruses, pathogenic microbes, lipoproteins, hormones, antibiotics and drugs, and a multi-immunobiosensor for measuring multicomponents concurrently.

Meanwhile, because liposome-analyte conjugate can be fabricated according to the embodiment, influence of the invention is very great. Namely, liposome-analyte conjugate instead of said cytolytic agent-analyte conjugate is fabricated and applied with an analyte in matrix, and then as result of immunochemical reaction, liposome-analyte conjugate passing through the competition point is ruptured by the cytolytic agent immobilized around electrode to generate signal.

As described above, immunobiosensor can be practically utilized according to the described embodiment.

Claims

1. An electrochemical immunobiosensor which comprises an electrode part where a plurality of electrodes are formed on an insulating base plate and a part of the electrode is exposed by selectively forming an insulating layer on the electrode; and a matrix part where a portion absorbed with analyte-cytolytic agent conjugates, a portion with an immobilized recepter which binds with the analyte, and a portion fixed with electroactive species-containing liposomes, to perform the functions of liposome-based signal amplification and immunochromatography; and said electrode portion exposed in the electrode part and the liposome portion in the matrix are integrally combined.

2. An electrochemical biosensor according to claim 1, wherein the matrix part is made of a material selected from a group consisting of filter paper of cellulose or nitrocellulose, polystyrene, polyvinyl and activated nylon film.

3. An electrochemical biosensor according to claim 1, wherein the electroactive species-containing liposome is made of phospholipid such as dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidylglycerol (DPPG), dimyristoulphosphatidylcholine (DMPC) or cholesterol.

4. An electrochemical biosensor according to claim 3, wherein the electroactive species entrapped in the liposomes are selected from a group consisting of ferrocene derivatives(e.g. ferrocene, dimethylferrocene), potassium ferrocyanide, viologen derivatives, 2,6-dichlorophenolindophenol (DCPIP), p-aminophenol, tetrathiafulvalene (TTF), tetracyanoquino-dimethane (TCNQ), phenazine methosulphate (PMS), Meldola's blue (7-dimethylamino-1,2-benzophenoxazine), 1,2-benzophenoxazine-7-one (BPO) and thionin.

5. An electrochemical biosensor according to claim 1, wherein the analyte-receptor binding to the analyte, at the site where the site absorbed with an analyte-cytolytic agent conjugate and the site with an immobilized receptor which binds to the analyte in the matrix, is a couple of physiologically bioactive materials such as antigen-antibody, biotine-avidin, immunoglobulin G-protein A, hormone-hormone receptor, DNA-DNA, RNA-RNA and drug-drug receptor.

6. An electrochemical biosensor according to claim 1, wherein the cytolytic agent is a physiologically bioactive material such as melittin or phospholipase C.

7. An multi-immunobiosensor for assaying multi-component which comprises overlapping a plurality of the electochemical immunobiosensors which comprises an electrode part where a plurality of electrodes are formed on an insulating base plate and a part of the electrode is exposed by selectively forming an insulating layer on the electrode; and a matrix part where a portion absorbed with analyte-cytolytic agent conjugates, a portion with an immobilized recepter which binds with the analyte, and a portion immobilized with electroactive species-containing liposomes; and said electrode portion exposed in the electrode part and the liposome portion in the matrix are integrally combined, with control sensor which generates reference signals to enable simultaneous measurement of physiologically important multi-component species.

8. An electrochemical biosensor according to claim 7, wherein the matrix part is made of a material selected from a group consisting of filter paper of cellulose or nitrocellulose, polystyrene, polyvinyl and activated nylon film.

9. An electrochemical biosensor according to claim 7, wherein the electroactive species-containing liposome is made of phospholipid such as dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidylglycerol (DPPG), dimyristoulphosphatidylcholine (DMPC) or cholesterol.

10. An electrochemical biosensor according to claim 9, wherein the electroactive species entrapped in the liposomes are selected from a group consisting of ferrocene derivatives(e.g. ferrocene, dimethylferrocene), potassium ferrocyanide, viologen derivatives, 2,6-dichlorophenolindophenol (DCPIP), p-aminophenol, tetrathiafulvalene (TTF), tetracyanoquino-dimethane (TCNQ), phenazine methosulphate (PMS), Meldola's blue (7-dimethylamino-1,2-benzophenoxazine), 1,2-benzophenoxazine-7-one (BPO) and thionin.

11. An electrochemical biosensor according to claim 7, wherein the analyte-receptor binding to the analyte, at the site where the site absorbed with an analyte-cytolytic agent conjugate and the site with an immobilized receptor which binds to the analyte in the matrix, is a couple of physiologically bioactive materials such as antigen-antibody, biotine-avidin, immunoglobulin G-protein A, hormone-hormone receptor, DNA-DNA, RNA-RNA and drug-drug receptor.

12. An electrochemical biosensor according to claim 7, wherein the cytolytic agent is a physiologically bioactive material such as or phospholipase C.

13. An electrochemical biosensor according to claim 7, wherein the same electroactive species-containing liposomes are immobilized on each matrix part of each immonobiosensor.

14. An electrochemical biosensor according to claim 7, wherein the control sensor only includes a matrix part immobilized with eletroactive species-containing liposomes.

FIG.1

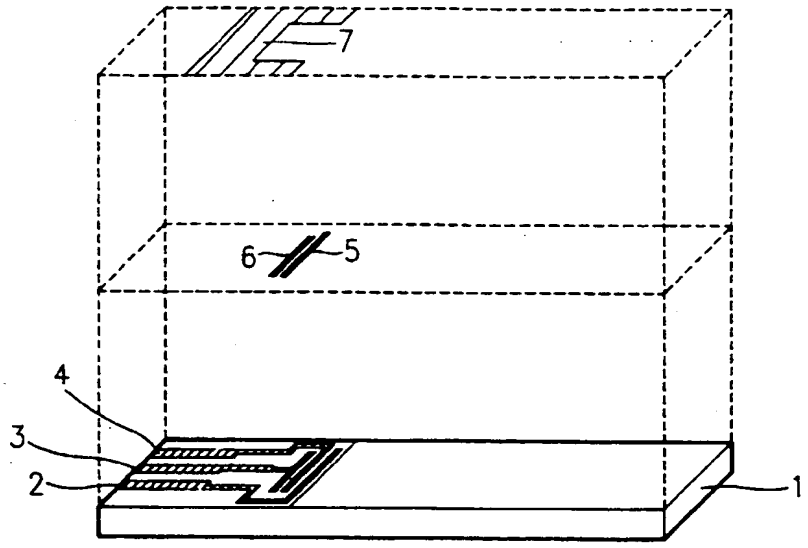


FIG.2

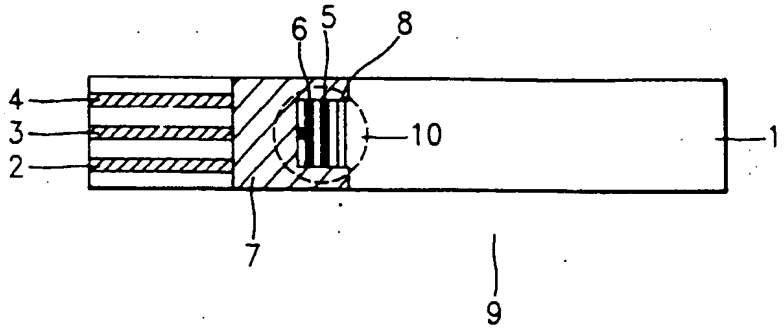


FIG.3

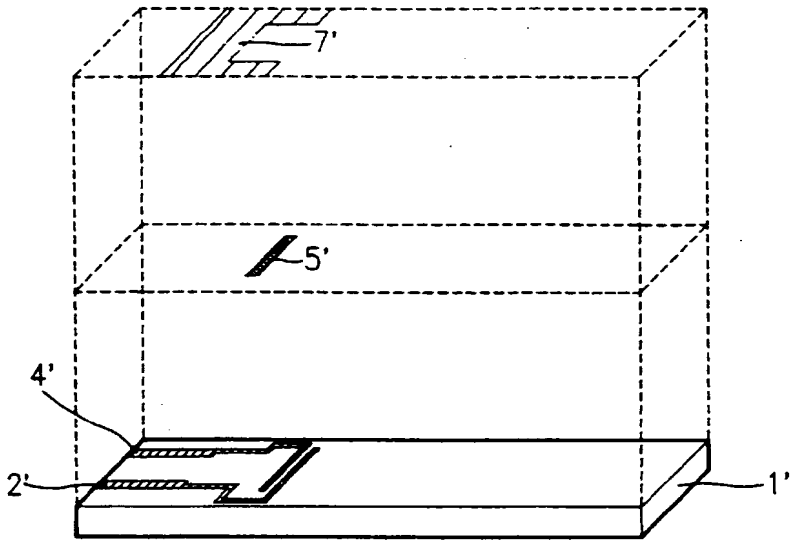


FIG.4

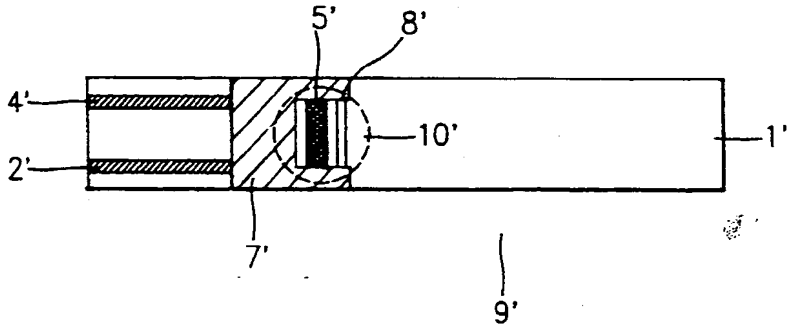


FIG.5

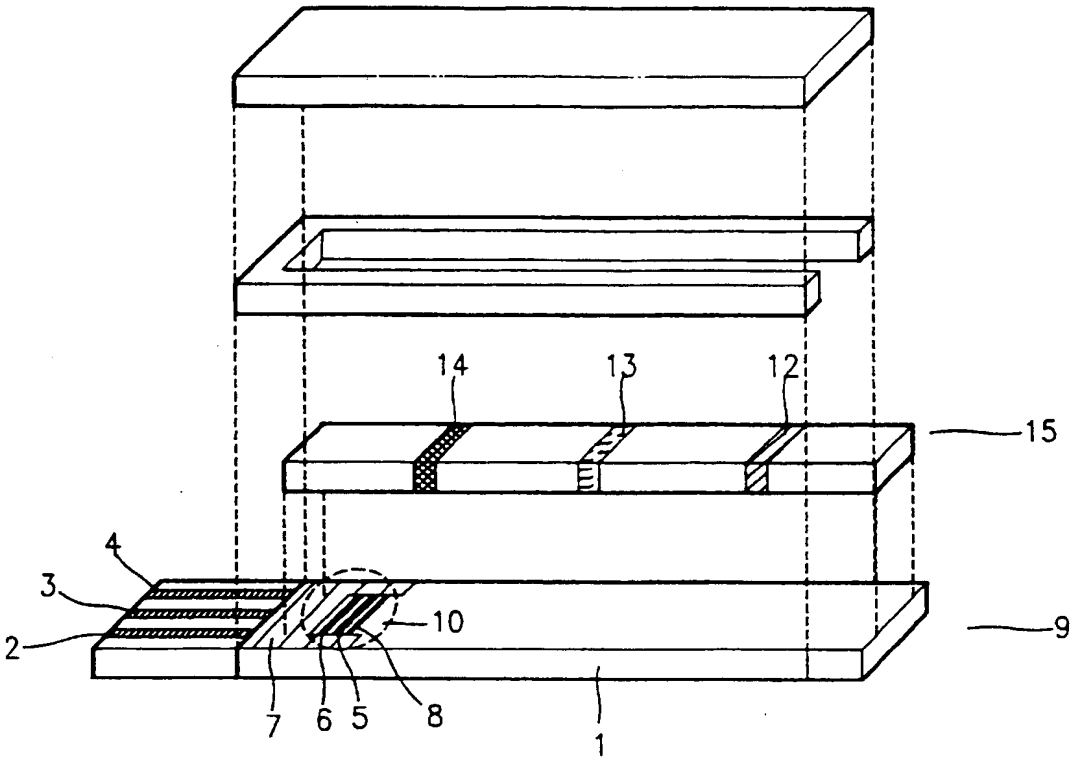
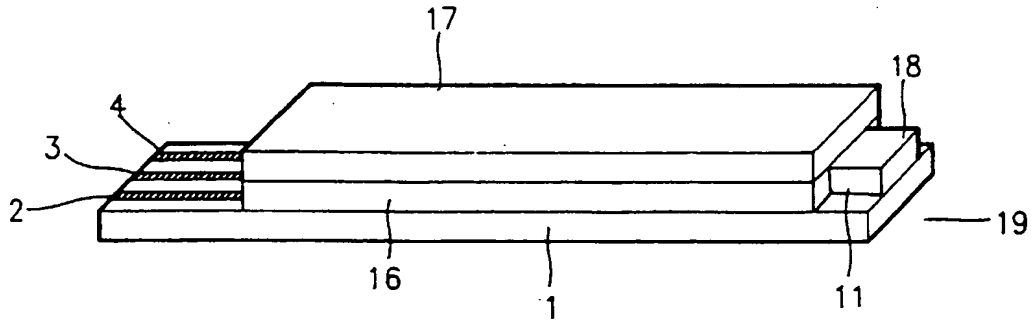


FIG.6



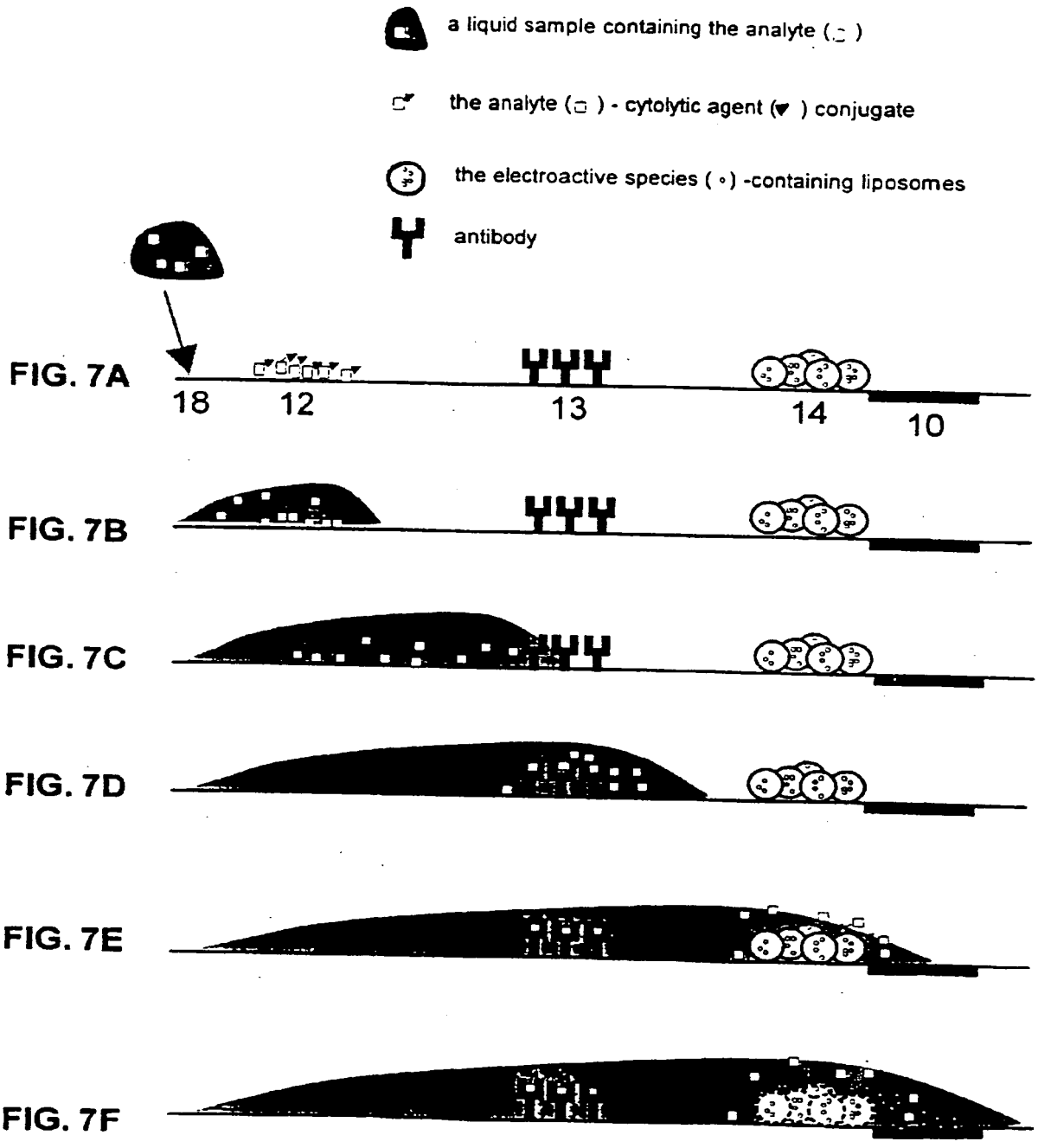


FIG. 7A

FIG. 7B

FIG. 7C

FIG. 7D

FIG. 7E

FIG. 7F

FIG.8

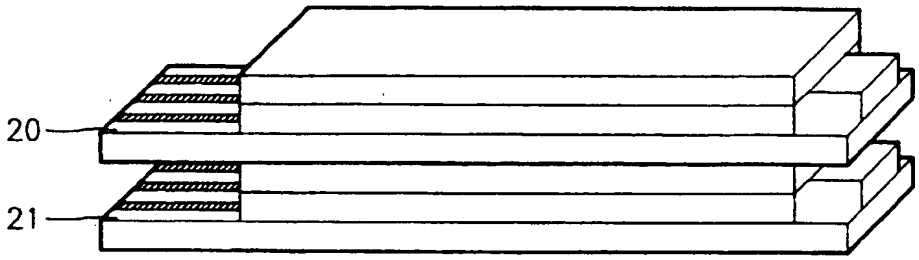
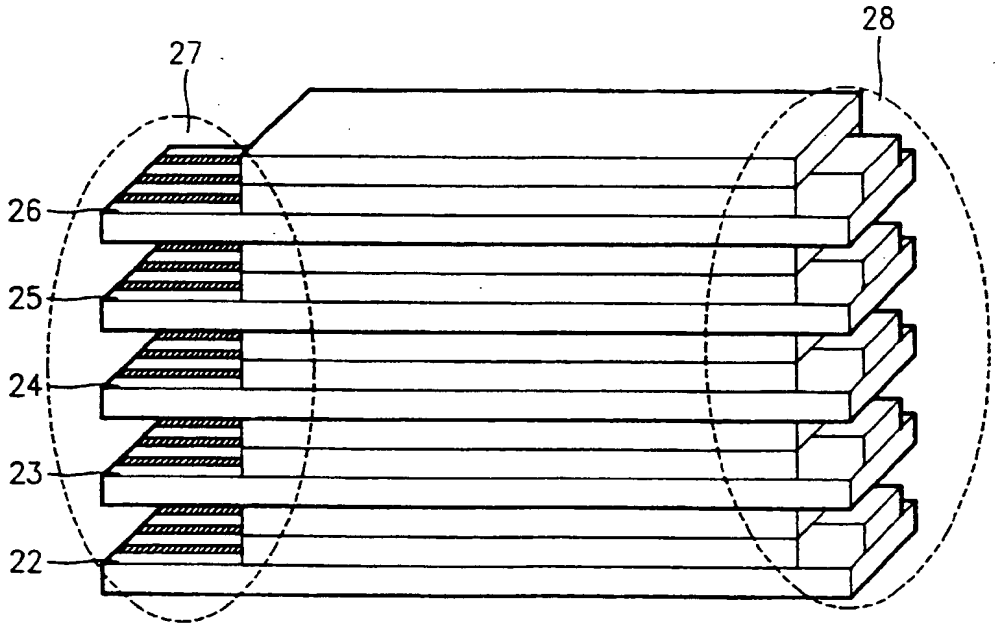


FIG.9



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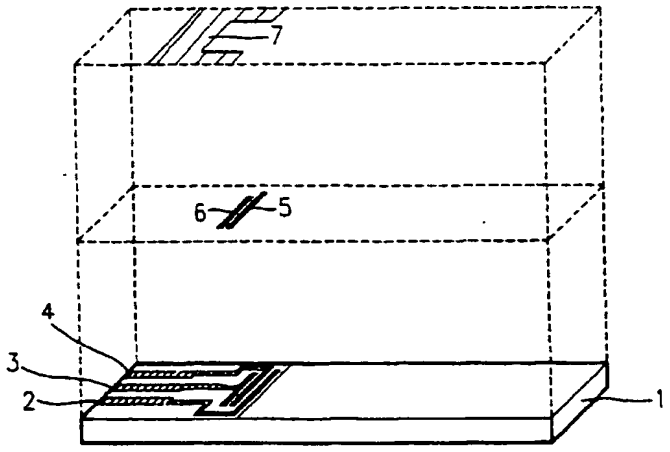
(54) Electrochemical immunobiosensor

(57) The present invention relates to an electrochemical immunobiosensor which comprises an electrode part where a plurality of electrodes are formed on an insulating base plate and a part of the electrode is exposed by selectively forming an insulating layer on the electrode; and a matrix part where a portion absorbed with analyte-cytolytic agent conjugates, a portion with an immobilized recepter which binds with the analyte, and a portion fixed with electroactive species-containing liposomes, to perform the functions of liposome-based signal amplification and immunochromatography; and said electrode portion exposed in the electrode part and the liposome portion in the matrix are integrally combined.

FIG.1



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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A	DE 36 18 101 A (HITACHI LTD) 4 December 1986 * the whole document *	1-14	G01N27/327 G01N33/543 G01N33/58
A	WO 91 16630 A (OPTICAL SYSTEMS DEV PARTNERS) 31 October 1991 * the whole document *	1-14	
A	EP 0 352 138 A (MEDISENSE INC) 24 January 1990 * the whole document *	1-14	
A	ANAL. CHEM. (1980), 52(11), 1610-13 CODEN: ANCHAM;ISSN: 0003-2700, 1980, XP000615736 SHIBA, KUNIO ET AL: "Thin-layer potentiometric analysis of lipid antigen-antibody reaction by tetrapentylammonium (TPA+) ion loaded liposomes and TPA+ ion selective electrode" * the whole document *	1-14	
A	ANAL. CHEM. (1988), 60(2), 142-7 CODEN: ANCHAM;ISSN: 0003-2700, 1988, XP000615737 KANNUCK, ROSANNE M. ET AL: "Measurement of liposome - released ferrocyanide by a dual-function polymer modified electrode" * the whole document *	1-14	
A	BIOTECHNOL. TECH. (1995), 9(6), 389-94 CODEN: BTECE6;ISSN: 0951-208X, 1995, XP000614545 KIM, H.-J. ET AL: "A novel liposome-based electrochemical biosensor for the detection of hemolytic microorganisms" * the whole document *	1-14	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)
G01N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	22 January 1997	Hoekstra, S

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